

WE CLAIM:

1. A method of producing a humanized mouse monoclonal antibody which consists of the steps:

- 5 (a) constructing a first human antibody heavy or light chain library in which each chain has at least one complementarity determining region (CDR) loop and each such loop is flanked by unaltered human framework residues and has the amino acid sequence of a corresponding mouse antibody heavy or light chain CDR  
10 loop;
- (b) constructing a second human antibody heavy or light chain library, in which each complementarity determining region (CDR) loop and each such loop is flanked by unaltered human framework residues and has  
15 the amino acid sequence of a corresponding mouse antibody heavy or light chain CDR loop, wherein the chains of said second library are the complementary heavy or light chain of said first library chains, such that one library is a light chain library and the other  
20 library is a heavy chain library;
- (c) creating a library of heavy and light chain pairs by combining chains from said library of step (a) with a complementary chain from an antibody which binds a preselected antigen forming a library of  
25 heavy and light chain pairs;
- (d) isolating antigen binding chains of step (a) by selecting from the library of step (c), a heavy and light chain pair which binds to said preselected antigen, isolating from the antigen binding heavy and  
30 light chain pair, the antigen binding chains of step (a);
- (e) creating a humanized pair library by combining isolated antigen binding chains of step (d), with chains of said library of step (b), so that a first

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isolated chain of step (d), together with a complementary second chain in said library of step (b) combine to form a heavy and light chain humanized pair library; and

5 (f) selecting from the humanized pair library of step (e) a humanized heavy and light chain pair that binds to said preselected antigen; and

(g) combining two of said selected humanized heavy and light chain pair of step (f) to form a whole  
10 antibody.

2. The method of claim 1 wherein the human library contains light chains and the complementary chain is a heavy chain.

3. The method of claim 1 wherein the human  
15 library contains heavy chains and the complementary chain is a light chain.

4. The method of claim 1 wherein the heavy chain is a Fd fragment.

5. A method of producing a humanized mouse  
20 monoclonal antibody heavy and light chain pair which consists of the steps:

(a) constructing a human light chain library wherein each said light chain has at least one complementarity determining region (CDR) loop that has  
25 an amino acid sequence corresponding to that of a mouse light chain CDR loop and each such loop is flanked by unaltered human framework residues;

(b) selecting a light chain from the light chain library of step (a), wherein the selection  
30 comprises combining a heavy chain from an antibody that binds a preselected antigen with a light chain from the library of step (a) to form a heavy and light chain pair library, screening said library for binding to said

preselected antigen, and isolating the light chains that bind to said preselected antigen;

5 (c) constructing a library of human heavy chains wherein each said heavy chain has at least one CDR loop that has an amino acid sequence corresponding to a mouse heavy chain CDR loop and each such loop is flanked by unaltered human framework residues;

10 (d) combining a chain from the heavy chain library of step (c) with a selected chain of step (b) to produce a second library of human heavy and light chain pairs; and

15 (e) selecting an antigen binding human heavy and light chain pair from the human library of step (d) by screening said library for binding with said preselected antigen and selecting those heavy and light chain pairs that bind said preselected antigen.

6. The method of claim 5 further comprising converting the selected heavy and light chain pair to whole antibody.

20 7. The method of claim 5 wherein the heavy chain of step (b) is a Fd fragment.

8. The method of claim 7 wherein the heavy chain Fd is a humanized mouse heavy chain fragment or a template mouse heavy chain fragment.

25 9. The method of claim 5 wherein only the light chain complementarity determining region three (LCDR3) loop from the mouse antibody is grafted onto the human light chain in place of the corresponding human light chain LCDR3 loop.

30 10. The method of claim 5 wherein only the heavy chain complementarity determining region three (HCDR3) loop from the mouse antibody is grafted onto the human heavy chain in place of the corresponding human heavy chain HCDR3 loop .

11. The method of claim 5 wherein in step (b) a humanized mouse heavy chain is used.

12. The method of claim 5 wherein only a light chain complementarity determining region three (LCDR3) loop from antibody LM609 produced from hybridoma cell line deposited with American Type Culture Collection under ATCC Accession No. HB 9537 is grafted in place of the LCDR3 loop of a light chain of step (a).

13. The method of claim 5 wherein only a heavy chain complementarity determining region three (HCDR3) loop from antibody LM609 produced from hybridoma cell line deposited with American Type Culture Collection under ATCC Accession No. HB 9537 is grafted in place of the HCDR3 loop of a heavy chain of step (d).

14. The method of claim 7 wherein the heavy chain Fd is a humanized mouse heavy chain.

15. The method of claim 7 wherein the heavy chain Fd is a template mouse heavy chain fragment.